

CRYPTOSPORIDIUM

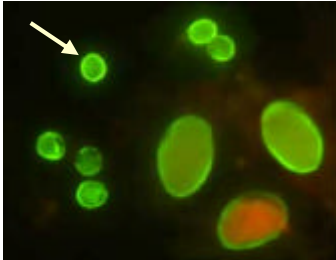
KEY POINTS FOR LABORATORY DIAGNOSIS I

BASIC GUIDELINES:

- a) **Multiple stool samples** (at least 3)* should be tested before a negative diagnostic interpretation is reported.
- b) To maximize recovery of oocysts, stool samples should be **concentrated** prior to microscopic examination (e.g., 10 min at 500 X *g* when using the formalin-ethyl acetate procedure). *Specimens to be used for EIA should NOT be concentrated because antigens are lost during the procedure!*
- c) **Choice of diagnostic techniques** depends on available equipment and reagents, experience, and considerations of time and cost. Useful diagnostic tests are as follows:

1. DIRECT FLUORESCENT ANTIBODY (FA) ASSAY:

Oocysts (4-6 μm) fluoresce apple green. This technique offers the highest combination of sensitivity and specificity and is considered the gold standard by many laboratories. However, it does not provide a permanent record such as a stained slide which can be archived. It requires special equipment (fluorescence microscope) and the purchase of commercially available test kits. The quality of reagents in commercially available test kits may be variable, or deteriorate under storage conditions, and controls are necessary to determine whether the kit is performing or not.



Oocysts of *C. parvum* (upper left arrow) and cysts of *Giardia intestinalis* (lower right) labeled with immunofluorescent antibodies.

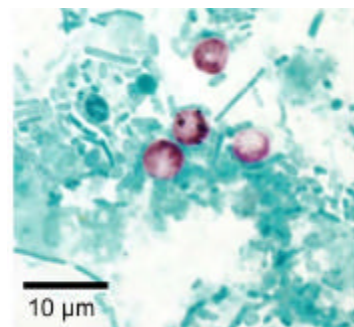
2. ENZYME IMMUNOASSAY (EIA):

The specimens should not be concentrated prior to testing! Antigens of *Cryptosporidium* in the feces are detected using this method. (Borderline positives and questionable negatives may be further confirmed by FA.) The EIA does not rely on microscopy skills, is highly sensitive and specific, and is useful for screening large numbers of specimens in a short time period. However, it may require special equipment (microplate reader) and the purchase of commercially available test kits. The quality of reagents in commercially available test kits may be variable, or deteriorate under storage conditions, and controls are necessary to determine whether the kit is performing or not.

3. KINYOUN'S MODIFIED ACID-FAST STAIN:

Oocysts (4-6 μm) often have distinct oocyst walls and stain from light pink to bright red. However, staining may be variable: in particular, infections that are resolving can have non-acid fast oocyst ghosts. Mature oocysts may have discernible sporozoites (up to 4).

Stool smear containing *Cryptosporidium parvum* oocysts stained with modified acid-fast technique.



*The concept of using multiple stool specimens is currently under review. Some studies have shown that the first sample is usually enough to provide accurate diagnosis in 90% of the cases.

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KEY POINTS FOR LABORATORY DIAGNOSIS II

KINYOUN'S MODIFIED ACID-FAST STAIN (CONTINUED): This method is the easiest, and most practical, and provides a permanent record. Misdiagnosis may result, however, due to the variability in staining and confusion with artifacts.

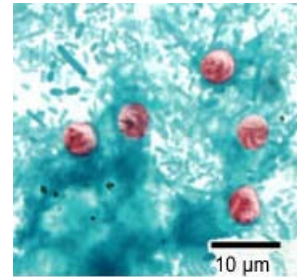
ADDITIONAL METHODS FOR DETECTION OF *CRYPTOSPORIDIUM*

(Oocysts may be detected by the following methods, but should be confirmed by the diagnostic techniques listed above.)

A. SAFRANIN STAIN:

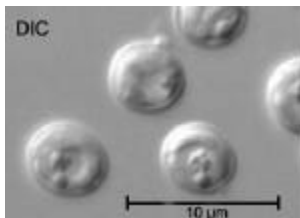
Oocysts of *Cryptosporidium* often (but not always) stain a bright reddish-orange color. This method, advocated for *Cyclospora*, is not widely used for *Cryptosporidium* because the *Cryptosporidium* oocysts may not always stain.

Stool smear containing *Cryptosporidium parvum* oocysts stained with safranin stain technique.



B. WET MOUNT:

In bright-field (differential interference contrast or DIC) microscopy, oocysts appear as small round structures (4 - 6 µm) similar to yeast. They do not autofluoresce. This method is less useful for *Cryptosporidium* than it is for *Cyclospora*, especially when low numbers of oocysts can be obscured by other fecal elements.



C. TRICHROME STAIN:

Oocysts appear as small round structures measuring 4 to 6 µm. Trichrome stain is the routine permanent staining technique for stool specimens in most laboratories, and laboratorians should be familiar with the appearance of *Cryptosporidium* stained with trichrome so that oocysts may be detected during routine examinations. This staining method is inadequate for definitive diagnosis because all oocysts will appear unstained.

DIFFERENTIATING OOCYSTS OF *CRYPTOSPORIDIUM* VS *CYCLOSPORA*

Size: *Cryptosporidium* 4-6 µm; *Cyclospora* 8-10 µm.

Sporozoites: may be discernible in *Cryptosporidium* oocysts; not seen inside *Cyclospora* oocysts.

Autofluorescence: absent in *Cryptosporidium*; present in *Cyclospora*.

Direct Fluorescent Antibody (FA) Assay: available for *Cryptosporidium*.

Enzyme Immuno Assay (EIA): available for *Cryptosporidium*.